

# Neutrophil disorders and their management

R Lakshman, A Finn

## Abstract

**Neutrophil disorders are an uncommon yet important cause of morbidity and mortality in infants and children. This article is an overview of these conditions, with emphasis on clinical recognition, rational investigation, and treatment. A comprehensive list of references is provided for further reading.**

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Neutrophils are phagocytic granulocytes that constitute an important component of the rapid “non-specific” immune defences. They, like other leucocytes, are derived from pluripotent stem cell progenitors in the bone marrow. Upon stimulation by colony stimulating factors, including stem cell factor, granulocyte-monocyte colony stimulating factor (GM-CSF), and granulocyte colony stimulating factor (G-CSF), phagocyte precursors proliferate and develop in the bone marrow to form mature segmented neutrophils. The first three stages of neutrophil maturation—myeloblast, promyelocyte, and myelocyte—involve young actively dividing cells. After the myelocyte stage, the cells lose their ability to divide and form metamyelocytes, band cells, and finally segmented polymorphonuclear neutrophils. In times of stress, corticosteroids, complement fragments, and catecholamines accelerate the release of mature neutrophils, as well as metamyelocytes and band cells, into the circulation. Chemotactic substances such as C5a, interleukin 8 (IL-8), monocyte chemotactic factor, leukotrienes, and bacterial peptides induce the migration of circulating neutrophils to sites of infection and inflammation. This extravasation involves selectin mediated rolling along the vascular endothelium, integrin mediated firm adhesion to the endothelium, and transendothelial migration through intercellular junctions to extravascular sites. Migrating neutrophils have a profoundly different shape and form from those seen in the circulation; progress through the tissues is thought to be mediated principally by the integrin CD11b/CD18 on the cell surface,<sup>1</sup> and might be regulated by soluble gradients (chemotaxis) or fixed gradients (haptotaxis)<sup>2</sup> of chemoattractants.<sup>3</sup>

Neutrophils have an array of surface receptors that enable them to bind to and ingest foreign particles and microbes by a process known as phagocytosis. This mechanism is greatly facilitated if microbes are coated with opsonic proteins such as immunoglobulins, complement fragment C3b, and mannan binding lectin, for which the neutrophil expresses multiple specific receptors. Phagocytosis is followed by

degranulation and respiratory burst activity and this leads to killing of the target as well as inflammatory changes in the tissues. The respiratory burst is an important pathway for microbial killing and involves the generation of superoxide, hydrogen peroxide, hydroxyl radical, and subsequently hypochlorous acid and chloramines. Degranulation involves release of the contents of the cell granules and contributes to “oxygen independent bactericidal activity”. If granulocyte activation persists, neutrophils release substances such as monocytic chemotactic protein that attract monocytes to the area. These in turn release monokines that enhance lymphocytic infiltration and also present antigens to T cells to produce cell mediated immune responses and promote T dependent humoral (B cell) responses.

Neutrophils have a short circulating life span (about eight hours) after leaving the bone marrow and then undergo apoptosis.<sup>4</sup> Anti-apoptotic signals generated by growth factors and cytokines can affect neutrophil survival and increase neutrophil numbers.<sup>5</sup>

Neutrophil disorders can result from a reduced number of cells or defective function. Neutropenias are a result of one or more defects in the differentiation or proliferation in the bone marrow, or increased peripheral destruction. Functional disorders include disorders of chemotaxis, adhesion, phagocytosis, and the respiratory burst. The extent to which such disorders are recognised and diagnosed, understood at the cellular and molecular level, and amenable to corrective or compensatory medical treatment is very variable. In considering disorders of neutrophils, it is worth bearing in mind that humans can survive quite prolonged periods without medical intervention with profound deficiency of specific (lymphocytic) immunity, but all would perish of infection within days of onset of absolute neutropenia.

## Disorders of neutrophil number: neutropenia

Neutropenia is an absolute reduction in the number of circulating neutrophils. Interpretation of peripheral blood neutrophil counts is rendered more difficult by the fact that these can change rapidly within an individual in response to non-specific changes such as exercise and heart rate. Reference ranges<sup>6</sup> vary with age and race; infants have lower neutrophil counts than older individuals, as do Africans compared with whites.<sup>7</sup> Neutrophil counts obtained by analysis of blood many hours after collection may show falsely low values. After infancy, absolute neutrophil counts (ANC)  $< 1.5 \times 10^9/\text{litre}$  are considered

Sheffield Institute for Vaccine Studies,  
Division of Child Health, University of Sheffield Children's Hospital, Sheffield S10 2TH, UK  
R Lakshman  
A Finn

Correspondence to:  
Dr Lakshman  
R.Lakshman@sheffield.ac.uk

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Table 1 Causes of neutropenia

Transient
Chronic
(1) Congenital neutropenias
Failure of production (bone marrow)
Severe chronic neutropenia
Cyclic neutropenia
Reticular dysgenesis
(2) Associated with syndromes
Primary immunodeficiencies: XLA, hyper-IgM
Glycogen storage disease (1b)
Shwachman diamond syndrome
Cardioskeletal myopathy
Onychotrichodysplasia
Antibody mediated neutropenias
Autoimmune neutropenia of infancy
Alloimmune neonatal neutropenia
Neonatal neutropenia owing to autoimmune disease in mother
Autoimmune neutropenia seen in association with primary specific immunodeficiencies
Idiopathic
Chronic benign neutropenia

XLA, X linked agammaglobulinaemia.

to be abnormally low and severe infections occur at values below  $0.5 \times 10^9$ /litre.

Transient states of neutropenia, often only noted on a single measurement, and not lasting for more than two weeks, are common in children and not usually associated with clinical problems. They are usually associated with viral infections and may therefore be the result of transient dysregulation of myelopoiesis or accelerated consumption. By contrast, neutropenia in neonates might be caused by exhaustion of bone marrow reserves and can be an important sign of severe generalised infection, such as septicaemia. Other causes of transient neutropenia include drugs such as cytotoxic agents and nonsteroidal anti-inflammatory agents. Neutropenia can also occur in babies born to mothers with pregnancy induced hypertension. The term chronic neutropenia is usually reserved for abnormally low absolute neutrophil counts lasting more than six months.<sup>8</sup> Children with severe chronic neutropenia (SCN) can be registered with the Severe Chronic Neutropenia International Registry.\* Chronic states of neutropenia (table 1) may in some cases have definable underlying genetic causes and are discussed at greater length here. They consist of three major groups, those with congenital causes (which can result from the failure of production and/or maturation of neutrophil precursors or can be associated with various syndromes), those caused by antibody mediated destruction of neutrophil or neutrophil precursors (immune), and those where the neutropenia is the sole abnormal finding and the pathogenesis unclear—so called chronic benign neutropenia. Increasingly, clinical and laboratory evidence supports the view that most children with chronic benign neutropenia have immune mediated neutropenia, although the demonstration of autoantibodies may be difficult in some cases.

The risk of infection in chronic neutropenia is inversely proportional to the absolute neutrophil count (including immature forms) and to the reserve of neutrophils present in the bone marrow. Conditions associated with

reduced bone marrow reserve of neutrophils and neutrophil precursors (such as Kostmann's syndrome) pose a greater risk for severe infections compared with those associated with normal reserve and increased peripheral destruction (such as autoimmune neutropenia of infancy). Although there is good correlation between the severity of neutropenia and occurrence of infections, there is considerable variation in infective symptoms for any given neutrophil range, within a patient population and also in a single individual, owing presumably to other compensatory immunological factors and to chance.

In our experience, children with neutropenia commonly present with malaise and lethargy (parents often describe their children as totally unwilling to get up and walk around), skin infections (cellulitis and subcutaneous abscesses), mucosal and respiratory infections (gingivitis, stomatitis, aphthous ulcers, periodontitis, perirectal abscess, pneumonia, and otitis media), and septicaemia. In one series, six of 23 girls with chronic neutropenia had a history of abscess or cellulitis affecting the labia majora.<sup>9</sup> The paucity of neutrophils may dampen the inflammatory response, reducing the ability to localise the infection and permitting rapid dissemination. Classic radiological features of pneumonia may be absent and there might be little or no abdominal tenderness even in the presence of ruptured viscus.

The most common causes of infections in individuals with chronic neutropenia are mainly endogenous flora: *Staphylococcus* spp, *Serratia marcescens*, *Klebsiella* spp, other Gram negative organisms and *Aspergillus* spp. They are not noted to have an increased susceptibility to viral or parasitic infections.

#### SEVERE CONGENITAL NEUTROPENIA (SCN)

Children with SCN are characterised by the early onset of life threatening infections, severe persistent neutropenia, and maturation arrest at the myelocyte/promyelocyte stage.

Most of the initial cases were reported from Sweden<sup>10</sup> and showed an autosomal recessive pattern of inheritance. However, subsequently, autosomal dominant<sup>11</sup> and sporadic forms have been recognised. In two of a series of 13 patients with SCN, Dong and colleagues<sup>12</sup> found mutations in the G-CSF receptor (GCSFR) gene that affected the maturation signalling function of the GCSFR. Previously, he and colleagues had described similar mutations in three children with SCN, two of whom later developed acute myeloid leukaemia.<sup>13</sup> It is possible that such mutations render the receptor defective and hyporesponsive to G-CSF in physiological doses.<sup>14</sup> However, in most children with SCN, mutations of this gene are not found and they might have a defect in other specific molecules in the G-CSF signal transduction pathway.<sup>12</sup> Ward *et al* have reported a novel point mutation in the extracellular domain of the G-CSF receptor in a patient with SCN who was hyporesponsive to recombinant human G-CSF (rhG-CSF).<sup>15</sup> An association with human major histocompatibility complex (HLA) B12 has also been described.<sup>16</sup>

\*The Severe Chronic Neutropenia International Registry (SCNIR) can be contacted by email at registry@u.washington.edu

*Clinical features*

The condition usually presents in the first few months of life with life threatening infections such as omphalitis, cellulitis, lymphadenitis, stomatitis, septicaemia, meningitis, respiratory infections, and peritonitis. These infections are commonly caused by *Staphylococcus aureus*, *Escherichia coli*, and pseudomonas species. Before the use of rhG-CSF, three quarters of patients with SCN died before 3 years of age and at a mean age of 2 years.<sup>17</sup> Since the use of G-CSF, life expectancy has increased significantly. However, some patients have gone on to develop acute myelogenous leukaemia (AML) or myelodysplasias (MDS). A recent paper has suggested a risk of just under 9% for the development of AML/MDS for patients with SCN who are on G-CSF.<sup>18</sup> This might be associated with mutations in the GCSFR gene<sup>13,19</sup>; eight of 16 patients with SCN and the mutation developed AML or MDS.<sup>20</sup> However, Bernard *et al* have suggested that such mutations might not be involved in the transformation into AML.<sup>21</sup> In addition, the predisposition of individuals with SCN to develop AML was noted before rhG-CSF became available.<sup>22,23</sup> Therefore, it appears that this kind of progression towards malignant transformation might be intrinsic to the natural history of some SCN cases and has become “exposed” by the prolonged survival of these patients on rhG-CSF treatment, rather than being a direct side effect of the treatment.

*Investigations and diagnosis*

Investigations reveal absolute neutropenia, which may be present from the 1st day of life, with neutrophil counts persistently below  $0.2 \times 10^9$ /litre of blood.<sup>10</sup> There may be associated monocytosis, eosinophilia, anaemia, and thrombocytosis. On bone marrow examination, there is myeloid hyperplasia with maturation arrest at the level of the promyelocyte/myelocyte stage. Bone marrow culture shows that the precursor stem cell in the bone marrow is capable of proliferating, with colonies of normal size and number, but these do not usually form mature neutrophils without exogenous G-CSF.<sup>24</sup>

## RETICULAR DYSGENESIS

This is a rare condition characterised by congenital agranulocytosis, lymphopenia, and lymphoid and thymic hypoplasia, leading to severe combined immunodeficiency.<sup>25</sup> It appears to be the result of a sporadically occurring defect in the development of haematopoietic stem cells. Affected children present with overwhelming infections in the 1st few days or weeks after birth. The full blood count shows neutropenia and lymphopenia. Lymphocyte subset analysis shows severe deficiency of all types of lymphocytes. The haemoglobin and platelet counts are normal. The bone marrow examination shows pronounced hypoplasia with absent myeloid and lymphoid elements. The differential diagnosis of reticular dysgenesis is severe combined immunodeficiency with maternal engraftment causing neutropenia through a graft versus host disease effect.

## CYCLICAL NEUTROPENIA

This is a rare blood disease characterised by regularly recurring symptoms of fever, malaise, mucosal ulcers, and occasionally life threatening infections related to regular cyclical fluctuations in numbers of blood neutrophils.

A pattern of autosomal dominant inheritance with variable expression is seen in up to 25% of cases.<sup>26</sup> It has been reported in identical twins.<sup>27</sup> The condition is caused by cyclical oscillations in the bone marrow production and release of mature neutrophils,<sup>28-30</sup> which might be associated with cyclical fluctuations in numbers of other blood cells such as monocytes, eosinophils, lymphocytes, platelets, and reticulocytes.<sup>31</sup> Bone marrow myeloid progenitors are present in high concentrations and the defect is thought to be caused by the inability of these precursors to respond to physiological concentrations of G-CSF.<sup>32</sup> In humans, transplantation of the defective bone marrow has resulted in transfer of the cyclic haematopoiesis to the recipient.<sup>33</sup> Recently, Horwitz *et al* have demonstrated mutations in the gene encoding neutrophil elastase (a 240 amino acid mature protein found in neutrophil granules) in a series of patients with cyclical neutropenia, and postulated that the condition is caused by perturbed interaction between neutrophil elastase and serpins or other substrates, which affects the timing of the biological clock governing haematopoiesis.<sup>34</sup>

*Clinical features*

Children with cyclic neutropenia nearly always present before 10 years of age; their clinical features may be characteristic with regular episodes of fever, malaise, mood swings, and oral ulcers lasting for three to six days and occurring at intervals of about three weeks. However, in our experience, the periodic nature of symptoms is often not obvious even on direct questioning of the patient. This is important because it means it is worthwhile looking for cyclical neutropenia in cases where the periodicity is not obvious and cyclical neutropenia is not initially demonstrated. Because these children are often repeatedly ill, it is not uncommon to find that full blood counts have been done repeatedly in the past and neutropenia often demonstrated but ignored because it is not persistent. The disorder does not appear to predispose to leukaemia or aplastic anaemia and, as these children get older, the condition tends to improve, although it can persist in adults. Although considered relatively benign, it always carries the risk of potentially life threatening infection; death occurs from overwhelming infection in as many as 10% of affected patients as a result of infections such as pneumonia, cellulitis, gangrene, or peritonitis.<sup>35-37</sup>

*Investigations and diagnosis*

The diagnosis is made by monitoring full blood counts twice a week for six weeks and identifying the characteristic cyclical changes in the neutrophil counts. In 70% of patients, there is a 21 day cycle, with severe neutropenia persisting for three to 10 days before recovering. In

others, the period can be as short as 15 days or as long as 35 days, but tends to stay fixed for each individual.<sup>38</sup> Patients also have characteristic values at which they cycle, with some cycling at very low values so that they only “reach” normality briefly every month. The bone marrow shows hypoplasia or maturation arrest during neutropenic episodes and hyperplasia during recovery.

#### ANTIBODY MEDIATED NEUTROPENIAS

These consist of two main groups, autoimmune neutropenia of infancy (AIN) and the antibody mediated neutropenias that occur in the neonatal period. Chronic benign neutropenia in childhood (CBN) is a label usually used for children whose symptoms clinically resemble those of AIN and most probably have antibody mediated neutropenia, but in whom antineutrophil antibodies cannot be demonstrated.<sup>9 39-41</sup> Such children may also have AIN but are probably reaching the end of their neutropenic period, when the antibodies disappear and neutropenia resolves. Autoimmune neutropenias and other cytopenias are also seen in association with some primary specific immunodeficiencies.

#### *Autoimmune neutropenia of infancy (AIN)*

This is the most common cause of chronic neutropenia in childhood and is characterised by the presence of antineutrophil antibodies.

The autoantibodies can mediate peripheral destruction of the neutrophils and/or inhibit myelopoiesis in the bone marrow. It has been suggested that antibodies directed against neutrophil precursors may produce a more severe neutropenia and clinical course than antibodies against mature neutrophils.<sup>42 43</sup> Boxer *et al* documented the presence of antineutrophil antibodies that disappeared with subsequent remission in patients with chronic neutropenia.<sup>44</sup> The reason why such antibodies are produced is not known and, in most patients, it is not associated with other autoimmune illnesses, although in some it may be associated with autoimmune haemolytic anaemia and/or thrombocytopenia. The disappearance of autoantibodies precedes the spontaneous normalisation of the neutropenia and remission of the condition.<sup>41</sup>

Bux *et al* reported the diagnosis and clinical course of 240 cases of autoimmune neutropenia of infancy.<sup>41</sup> They found that the average age at diagnosis was 8 months. Eighty percent of patients only suffered from mild infections such as pyoderma, otitis media, and infections of the upper respiratory tract. In 8% of cases, AIN was detected by chance, and 12% suffered severe infections such as pneumonia, meningitis, and sepsis. In most patients, the neutropenia lasted seven to 24 months and then spontaneously resolved.

The full blood count shows absolute neutropenia, although there is often non-cyclical random fluctuation in neutrophil numbers from zero to near normal. There may be associated monocytosis, eosinophilia, anaemia, and thrombocytosis. The diagnostic feature is the presence of antigranulocyte antibodies. The

best antibody screening procedure is a combination of granulocyte immunofluorescence and agglutination techniques,<sup>45</sup> which in combination detect antineutrophil antibodies in almost all such patients. Antibody titres may be low and screening for granulocyte specific antibodies in patient's serum may have to be repeated several times.<sup>41</sup> A positive test for granulocyte antibody can be crucial in the diagnosis of AIN and can render bone marrow biopsy unnecessary. Bone marrow examination reveals a normocellular or hypercellular marrow with myeloid hyperplasia.

SCN often presents with infections in the first month of life, while AIN usually presents later. The incidence and severity of bacterial infections is usually much less in AIN than in SCN. AIN may be mistaken for cyclic neutropenia because it shows day to day fluctuations of neutrophil counts. The main feature differentiating AIN from both SCN and cyclic neutropenia is the presence of granulocyte antibodies. Such antibodies can also be found in alloimmune neonatal neutropenia (see below), but in those cases, the antibodies do not last beyond 6 months of age and similar antibodies are detectable in the maternal serum.

#### *Immune neutropenia in the neonate*

Neutropenia in the neonate may be caused by antineutrophil antibodies that are transplacentally transferred. Maternal antineutrophil antibodies may be present in association with autoimmune disease, such as systemic lupus erythematosus or rheumatoid arthritis (neonatal maternal autoimmune neutropenia), or might be the result of prenatal maternal sensitisation by fetal neutrophils (alloimmune (isoimmune) neonatal neutropenia; ANN).<sup>46 47</sup> In ANN, the antibodies are most commonly directed against paternal NA1 or NA2 (neutrophil antigen 1 or 2), which are inherited by the fetus; such antibodies can remain detectable in the infant for up to six months. Most infants with alloimmune neonatal neutropenia are asymptomatic; some present with omphalitis, delayed separation of the cord, mild skin infections, fever, or pneumonia in the 1st weeks of life. The diagnosis of alloimmune neutropenia is confirmed by the demonstration of antineutrophil antibodies both in the baby and in the mother.

#### *Chronic congenital neutropenia in association with syndromes and metabolic conditions*

Congenital neutropenia is seen in several syndromes and metabolic conditions and may cause considerable additional morbidity in these children. The diagnosis and management of the neutropenia might improve the quality of life of these children.

*Glycogen storage disease type 1b (GSD 1b)*—Clinically, this condition is identical to the classic von Gierke glycogen storage disease (GSD 1a), but in addition almost all these children suffer from neutropenia and recurrent infections, including oral lesions and perianal abscesses.<sup>48 49</sup>

*Shwachman-Diamond syndrome*—This is an autosomal recessive condition characterised by

pancreatic insufficiency and neutropenia.<sup>50</sup> The absolute neutrophil count is generally between  $0.2$  and  $0.4 \times 10^9$ /litre. Other features include metaphyseal dysostosis and chronic eczema.

*Cardioskeletal myopathy*<sup>51-53</sup>—This X linked recessive condition is a relatively common cause of dilated cardiomyopathy and neutropenia in boys during infancy and early childhood. It can present as isolated neutropenia without clinical evidence of cardiac disease. Examination also reveals weakness in skeletal muscles.

*Onychotrichodysplasia*—This is an autosomal recessive condition characterised by sparse hair, hypoplastic nails, and persistent neutropenia.<sup>54</sup>

#### APPROACH TO DIAGNOSIS IN CHRONIC NEUTROPENIA

The urgency of investigations is dictated by age, clinical presentation, past history, and examination findings. Asymptomatic patients with isolated neutropenia can be observed clinically for several weeks, whereas those in whom the history and examination findings suggest the possibility of a serious underlying problem need to be evaluated urgently and completely. History should include any family history of immunodeficiency including indirect evidence such as unexplained deaths, repeated infections or known neutropenia, past history of infections, and history of drug ingestion. Examination should include the evaluation of growth and development, careful examination of skin and mucous membranes, upper and lower respiratory tract, and palpation for lymphadenopathy and hepatosplenomegaly. The following tests should be considered.

(1) Repeated full blood counts will help differentiate transient, cyclical, and chronic neutropenias. This will also differentiate isolated neutropenia from that associated with anaemia and/or thrombocytopenia and may point to associated immunological or oncological disease.

(2) Neutrophil antibody assays are usually performed in specialist laboratories and require separate analysis of neutrophils and serum from the patient. They are positive in cases with AIN and ANN.

(3) Bone marrow aspiration is useful to rule out aplastic anaemia, leukaemia, and infiltration by another malignancy, such as neuroblastoma. Although the bone marrow picture is usually characteristic in SCN and reticular dysgenesis, some authorities maintain that it is difficult to distinguish reliably between the absence of mature forms resulting from maturation arrest in SCN and autoimmune destruction in AIN.

(4) Serum immunoglobulin estimations are an essential part of the investigation of patients with neutropenia. Persistent or cyclic neutropenia is a well recognised association of hyper-IgM syndrome (CD40 ligand or gp39 deficiency).<sup>55-56</sup> Up to one third of boys with X linked agammaglobulinaemia (XLA) may also have neutropenia.<sup>57</sup> In other patients with neutropenia, polyclonal raised immunoglobulin values may reflect the chronic infections they experience.

(5) Lymphocyte subsets should be measured to exclude reticular dysgenesis in infants.

(6) An autoantibody screen is necessary to rule out systemic lupus erythematosus and other autoimmune conditions.

(7) Specific tests should be carried out if a syndromic or metabolic disorder is suspected.

#### MANAGEMENT OF CHRONIC NEUTROPENIA

Management depends on the severity of neutropenia, clinical course in the individual, and the aetiology of the chronic neutropenia.

##### *General measures*

Effective management to prevent and treat infections is essential in all cases of chronic neutropenia. It is thought that good mouth care, dental hygiene, and skin care can reduce recurrent infections. Vigilance for the emergence of serious infection and, when it is suspected, early and vigorous parenteral treatment with broad spectrum antibiotics after obtaining blood and other appropriate cultures is essential in all cases.

##### *Prophylactic antibiotics*

Regular prophylactic cotrimoxazole can be a simple but effective intervention in children with chronic neutropenias.<sup>41-58</sup> As in other forms of immunodeficiency, the decision to intervene in this way is influenced by both the clinical picture and pathology results, including the severity of neutropenia.

##### *rhG-CSF*

Komiyama *et al* were the first to explore using colony stimulating factor purified from urine to treat patients with chronic neutropenia.<sup>59</sup> Bonilla *et al* administered rhG-CSF to five patients with SCN.<sup>60</sup> All five patients showed a response and maintained neutrophil counts of  $> 1.0 \times 10^9$ /litre for more than nine months with subcutaneous maintenance treatment. Several subsequent studies have confirmed the effect of this treatment on the neutrophil count in children with SCN and have also shown that it results in the resolution of pre-existing infections, reduced numbers of new infections, and significant improvement in survival and quality of life.<sup>61-64</sup> Bonilla *et al*<sup>65</sup> published data on the long term safety of rhG-CSF in patients with chronic neutropenia. Their study comprised 54 patients, who were followed up while being treated with regular rhG-CSF for four to six years. The dose required to maintain ANC over  $1.0 \times 10^9$ /litre ranged from 0.8–60  $\mu\text{g}/\text{kg}/\text{day}$ . Only in three patients was there any evidence of refractoriness to treatment; one of these stopped treatment, whereas in the other two, a response was seen with further increases in dose. The higher doses needed in SCN may be useful to differentiate it from chronic benign neutropenia.<sup>66</sup> The decision on whether to start continuous rhG-CSF in children with cyclical neutropenia can be difficult and in some cases intermittent use to cover the troughs may be feasible.<sup>64-67-73</sup> Such treatment reduces the duration of the cycles and increases the mean neutrophil nadir,<sup>74</sup> and is associated with a pronounced improvement in the symptoms

and frequency of infections. Although daily administration of rhG-CSF increases neutrophil counts to normal within two weeks<sup>75-77</sup> in children with CBN/AIN, in association with clinical improvement and a reduction in the frequency of new infections,<sup>8</sup> most authorities will be influenced considerably by the clinical picture in deciding which children to treat. rhG-CSF has also been used in neonatal neutropenia of various aetiologies<sup>47, 78</sup> and in treating the neutropenia associated with GSD 1b and Shwachman-Diamond syndrome. Side effects thought to be the result of treatment included osteoporosis/osteopenia, bone pains, hepatomegaly, haematuria, thrombocytopenia, and splenomegaly (observed radiologically but not clinically).

#### Intravenous immunoglobulin treatment (IVIG)

In those diagnosed to have antibody mediated neutropenia and suffering recurrent or serious infections, treatment measures available include  $\gamma$ -globulin infusions. Although IVIG can be used to correct neutrophil counts, the effect is short lived and this approach is only used occasionally—for example, as an adjunctive approach in severe and life threatening infections,<sup>79-82</sup> unless the child has an associated disorder of antibody production.

#### Granulocyte transfusions

Granulocyte or buffy coat transfusions have been used as an adjuvant management strategy in individuals with chronic neutropenia and focal bacterial or fungal infections not responsive to usual treatment. Transfusion of paternal neutrophils that lack the sensitising antigen have been described in the treatment of neonates with alloimmune neutropenia with sepsis. However, the safety and effectiveness of this treatment modality are unknown.

#### Bone marrow transplantation (BMT)

In patients with SCN who do not respond to rhG-CSF, bone marrow transplantation remains an option.<sup>83</sup> Without an early HLA

matched bone marrow transplant, all children with reticular dysgenesis would succumb to overwhelming infection.<sup>84</sup>

### Disorders of neutrophil function

#### CHRONIC GRANULOMATOUS DISEASE (CGD)

CGD is a rare disorder characterised by absent or reduced function of the respiratory burst, which produces oxygen free radicals important for intracellular killing.

CGD is caused by congenital defects in the five components of the enzyme NADPH oxidase (fig 1), which catalyses the respiratory burst. The mutations underlying such defects are heterogenous. In about two thirds of cases, CGD occurs as an X linked recessive disease caused by a mutation in the glycoprotein 91 phagocyte oxidase gene (gp91 phox gene), which encodes a membrane bound subunit of the enzyme; the remaining one third of cases are inherited in an autosomal recessive manner and are caused by defects in the cytosol components p47phox (25%), p67phox (5%), or the smaller membrane bound subunit, p22phox (5%). In general, patients with p22phox and p67phox deficiency CGD have a similar clinical phenotype to those with X linked 91phox deficiency, but patients with 47 phox deficiency follow a milder course.<sup>85-88</sup> Patients with X linked CGD can, on the basis of genotypic analysis, be divided into the X91<sup>0</sup> (complete deficiency), X91- (partial deficiency), or X91+ (stable but inactive gp91 phox) subgroups.<sup>89</sup>

#### Clinical features

Most children with CGD present in the 1st year of life with recurrent bacterial and fungal infections.<sup>90</sup> The most frequently encountered pathogens are *S aureus*, *Aspergillus* spp, enteric Gram negative bacteria (including *S marcescens* and various salmonella species), and *Burkholderia cepacia*. Catalase negative organisms (such as *Streptococcus pneumoniae* and *Haemophilus influenzae*) are less frequent pathogens in children with CGD; the hydrogen peroxide they produce may be converted to hypochlorous acid by neutrophil myeloperoxidase and used to kill the bacteria. Infections include pneumonia, cutaneous infections (including perirectal abscesses), lymphadenitis, liver abscess, osteomyelitis, septicaemia, and otitis media. One of the hallmarks of the condition is the presence of granulomas caused by the chronic inflammatory response to the pathogen. These can mimic Crohn's disease or can present with obstructive gastrointestinal or urinary symptoms. Features commonly found on clinical examination include failure to thrive, hepatosplenomegaly, lymphadenopathy, and anaemia.

Finn *et al* reviewed 31 patients followed up between 1964 and 1989; the actuarial analysis showed 50% survival to the 3rd decade of life.<sup>91</sup> With newer treatment approaches, the prognosis may be better, particularly for those with CGD caused by 47 phox deficiency.<sup>89</sup>

Carriers of autosomal recessive forms of CGD are asymptomatic. Approximately half of X linked carriers suffer from recurrent

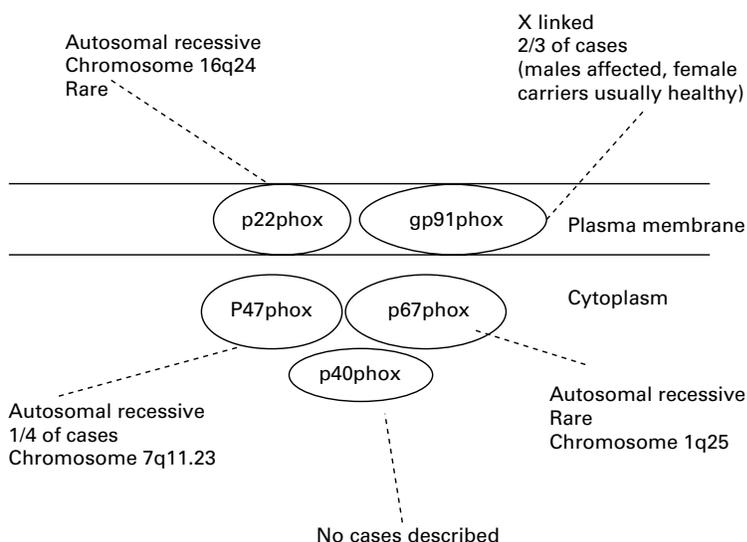


Figure 1 Components of NADPH oxidase and subgroups of chronic granulomatous disease.

stomatitis and/or gingivitis and about 25% develop discoid lupus erythematosus on sun exposure. A very small proportion of X linked CGD carriers may suffer from increased, although mild, infections.

#### Investigations

The diagnosis of CGD can be made at different levels. The bacterial killing test is a comprehensive screening test for defects in opsonisation, phagocytosis, or intracellular killing. It looks at the ability of phagocytes to kill catalase positive bacteria, such as *S aureus*, in vitro. However, it is technically difficult and time consuming to perform. The phorbol myristate acetate (PMA) nitroblue tetrazolium (NBT) slide test is an extremely easy and reliable screening test for disorders of oxidative metabolism. It detects all but the most mild X linked CGD carriers and variants.<sup>92</sup> Neutrophils are exposed to a stimulus, such as PMA, incubated with NBT, and staining assessed by counting 100 neutrophils on a smear. After stimulation, more than 95% of neutrophils from healthy individuals stain positive, with a blue black precipitate of formazan granules, whereas < 5% of neutrophils are stained in individuals with CGD. Carriers of X linked CGD usually show 30–70% positive cells. However, the test may give falsely positive/normal results in certain mild forms of CGD. More sensitive quantitative tests to measure respiratory burst activity in stimulated neutrophils using flow cytometric analysis are now available.<sup>93</sup> Neutrophils are stimulated in vitro using reagents such as PMA and the superoxide or hydrogen peroxide generated is measured. In one assay, hydrogen peroxide generated by stimulated neutrophils converts dihydrorhodamine to rhodamine and this is detected using a fluorescent detector. Other methods, generally used in research rather than clinical diagnosis, involve the measurement of oxygen consumption using an oxygen electrode, or estimating superoxide generation by the reduction of ferricytochrome c, or by hydrogen peroxide production by oxidation of homovanillic acid. Chemiluminescence with luminol can also be used as a sensitive test of NADPH oxidase activity.

The diagnosis of suboptimal respiratory burst dysfunction leads to further investigations to identify the subgroup of CGD. Western blot analysis using antibodies against each of the four NADPH oxidase components can show whether one of the components is missing. The absence of 47 phox protein and 67 phox protein is indicative of a gene mutation; however, for the membrane bound components, if one (either the 91 kDa or the 22 kDa phox protein) is missing the other will be absent too because all the subunits are needed for stabilisation and full expression. In such cases, it is useful to confirm or refute carrier status in the mother using the NBT slide test or dihydrorhodamine flow cytometry assay. However, failure to find that the mother is an X linked carrier does not completely rule out X linked CGD; such defects can occur as new mutations.

If all four subunits are detectable on western blot in an individual with respiratory burst dysfunction, the diagnosis may be a so called “+variant” of CGD. These are conditions where the synthesis of the protein is normal but the enzyme activity is lost. In such cases, cell free oxidase assays are used to differentiate defects in the cytosolic components from those affecting the membrane components. Some individuals have partial loss of 91 phox and proportional loss of NADPH oxidase activity; they are referred to as “-variants”.

Further tests can be done to identify the defect at the DNA level by mutation analysis; for autosomal recessive forms, this is the best way to identify carrier status. Mutations may involve deletions, insertions, splice site mutations, missense mutations, or nonsense mutations. Prenatal diagnosis of CGD can be made by the analysis of DNA from chorionic villous sampling or amniotic fluid cells if the specific mutation in the family has previously been identified.<sup>94</sup>

#### Management

Management involves measures to reduce the frequency of infections and to ensure their prompt recognition and treatment. Avoidance of BCG vaccination is advocated; children must receive all other routine immunisations and a yearly influenza vaccine. Oral and perianal hygiene are important and regular dental care, including antibacterial mouth washes, can help prevent gingivitis and periodontitis. Injuries must be washed well and rinsed with antiseptic solutions. Regular prophylaxis with cotrimoxazole can reduce bacterial infections.<sup>58</sup> The evidence is less clear about antifungal prophylaxis,<sup>95–96</sup> but itraconazole is often used in severe cases or where invasive fungal infection has occurred previously. Gallin *et al* showed that prophylactic treatment with  $\gamma$  interferon (IFN- $\gamma$ ) can reduce infection rates in patients with CGD.<sup>85–97–98</sup> However, the infection rates in the placebo control group in that study were higher than those generally seen in the UK where daily antimicrobial (cotrimoxazole) prophylaxis is routine. Accordingly, in most UK centres IFN- $\gamma$  prophylaxis is offered only in selected cases. IFN- $\gamma$  does not correct the defective metabolism in CGD but may work by augmenting nitric oxide production in neutrophils.<sup>99</sup> Once infection occurs, this must be treated with appropriate parenteral antibiotics in sufficient dosage and duration to eradicate the organism; often abscess formation will need surgical measures. Although corticosteroids should be generally avoided in CGD, they are useful in low doses in the management of symptomatic complications of granuloma formation at different sites.<sup>100–101</sup> Results of bone marrow transplantation have been much better in the past decade (using matched siblings as donors), although numbers are still limited, and there are reports of successful outcomes even in the most severely infected patients.<sup>102</sup>

## OTHER DISORDERS OF INTRACELLULAR KILLING

*Myeloperoxidase (MPO) deficiency*

This is a minor phagocyte intracellular killing defect caused by deficiency of the MPO enzyme. It is common, with an incidence of between 1/2000–4000 in the general population.<sup>103</sup> It is caused by a mutation in the MPO gene on chromosome 17. Granulocytes with MPO deficiency have defective generation of hypochlorite ion, but have normal superoxide ion production and hence intracellular killing of phagocytosed microbes is only minimally affected. In some individuals, in vitro assays show slightly reduced killing of *S aureus* and, more importantly, impaired killing of *Candida albicans*. Most individuals with MPO deficiency are completely asymptomatic; those who suffer from recurrent or severe candidiasis may also have diabetes mellitus or other compromises in immune function. The diagnosis is usually made by appropriate histochemical staining of the neutrophils: some modern haematology automated cell counters can be set to detect low MPO values.

*Glutathione synthetase deficiency*

This is a very rare condition affecting the production of glutathione, which is a potent antioxidant found in most cells, including granulocytes. Affected children have subnormal respiratory burst function and intracellular killing. Children present with haemolytic anaemia and recurrent otitis. The diagnosis is confirmed by demonstrating absent or low glutathione synthetase values in red blood cells.

*Severe glucose-6-phosphate dehydrogenase**(G6PD) deficiency*

White individuals with severe (< 1%) G6PD activity can present with clinical features similar to CGD. This is not seen in Asians or black individuals with similar severe enzyme deficiency. G6PD is important for ensuring the adequate availability of NADPH for the respiratory burst. Affected children present with recurrent infections similar to CGD. In addition, they are prone to haemolysis and may suffer recurrent episodes of severe anaemia and jaundice. Investigations show a raised reticulocyte count and absent NBT dye reduction in almost all neutrophils. The diagnosis is confirmed by demonstrating undetectable red blood cell and leucocyte G6PD.

## LEUKOCYTE ADHESION DEFICIENCY

The process of neutrophil migration from the blood stream through the endothelium is mediated by various families of adhesion molecules and their ligands. Integrins are transmembrane cell surface proteins present in all leucocytes and are essential for firm adhesion of the leucocyte to the endothelium and subsequent transendothelial migration. Each integrin molecule is a heterodimer consisting of an  $\alpha$  and a  $\beta$  chain. Selectins are another family of adhesion molecules that mediate the initial transient “rolling” interaction between leucocytes and endothelium. Characteristically, their counterligands are glycosylation moieties (hence the name lectin) that decorate counter-

receptors. An example is the carbohydrate blood group antigen, sialylated Lewis X (SLeX).

The term leucocyte adhesion deficiency (LAD) was first proposed by Anderson and Springer in 1987<sup>104</sup> for a condition caused by a defect of the  $\beta 2$  integrins, heterodimers of  $\alpha$  and  $\beta$  subunits called CD11 and CD18, respectively. Three different  $\alpha$  subunits CD11a, CD11b, and CD11c combine with a common  $\beta$  subunit CD18. The molecular defect results from absent or diminished  $\beta 2$  subunit (CD18) caused by mutations that can be mapped to chromosome 21q21.3.<sup>104</sup> This leads to a lack of functional CD11/CD18 glycoproteins in all leucocytes. The neutrophils in this condition were able to roll along endothelial cells but were unable to adhere firmly to them or emigrate to the tissues. Thus, neutrophils do not accumulate at extravascular inflammatory sites, despite pronounced peripheral blood leucocytosis. Subsequently, another similar condition, LAD 2 was described, where the defect is one of addition of fucosyl groups. A variant form of LAD 1, in which  $\beta 2$  integrins are expressed but not functional, has also been described.<sup>105 106</sup>

*LAD 1*

Inheritance is autosomal recessive. More than 600 cases have been described and present with recurrent bacterial infections from birth. Infections mainly affect the skin and mucous membranes; omphalitis and delayed separation of the cord (often beyond 21 days<sup>107</sup>) is a common presentation. Infected sites often undergo necrosis but characteristically there is no pus, even though there is pronounced peripheral blood neutrophilia. Chronic skin ulcers without pus can form and there is delayed wound healing. Recurrent subcutaneous and mucosal infections (perirectal abscesses, pyoderma gangrenosum,<sup>108</sup> otitis media, pharyngitis, ulcerative stomatitis, gingivitis, and periodontitis) are seen. Life threatening infections such as septicaemia, bronchopneumonia, and aseptic meningitis can occur; more than 75% of these children die before the age of 5 years if they do not receive a bone marrow transplant. The most frequent bacteria involved are *S aureus* and Gram negative enteric organisms. Patients also suffer from fungal infections but do not have increased susceptibility to viral infections. The severity of infections and complications is related to the severity of CD18 deficiency; cases with < 1% expression are clinically severe, whereas those with 2.5–10% expression are moderate to mild. LAD 1 carriers have 50% expression of CD18 and are clinically asymptomatic.

The diagnosis is to be suspected in any infant with serious infections accompanied by striking neutrophilia in the peripheral blood. Absence of neutrophilia in a newborn infant who has delayed cord separation but is otherwise well generally rules out the diagnosis.<sup>109</sup> The white blood cell count ranges between 15 and 160  $\times 10^9$ /litre and 50–90% of these are neutrophils. The diagnosis can be confirmed by flow cytometric analysis of

peripheral blood neutrophils using monoclonal antibodies for CD11 or CD18.<sup>110-111</sup> In our laboratory, we routinely measure the expression of CD11a and CD11b  $\alpha$  chains and CD18  $\beta$  chains and also assess modulation of expression after stimulation with *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP). In vitro functional studies, if performed, show reduced chemotaxis, reduced adherence to endothelial monolayers, and abnormal granulocyte aggregation.

Leucocytes express CD18 at 20 weeks gestation and at this time blood can be obtained by cordocentesis for prenatal diagnosis. Mutational analysis on chorionic villous biopsy or cells obtained by amniocentesis may be possible when the precise familial mutation is known.

All patients with severe LAD 1 need bone marrow transplantation.<sup>112</sup> Patients with mild to moderate deficiency may be managed with prophylactic antimicrobial treatment to prevent infections and measures to ensure their prompt recognition and treatment.

### LAD II

LAD II has been described in only three unrelated boys. A recent paper<sup>113</sup> describes the third patient, who was investigated extensively, and found to have severe hypofucosylation of glycoconjugates bearing fucose in different glycosidic linkages in all types of cells. The authors suggest that this is not only a disorder of leucocytes but a generalised metabolic disease affecting the metabolism of fucose. It is associated with severe mental retardation and short stature and presents with frequent infections similar to the moderate to mild phenotype of LAD I. These children too had reduced signs of inflammation with infections and no pus formation in spite of pronounced peripheral blood neutrophilia. These children also have the Bombay blood group (absence of the H antigen) and this is a useful clue; confirmation of diagnosis needs cell sorter analysis for SLex (CD15) expression on leucocytes. There was a normal density of CD11/CD18 on their leucocytes and this could be increased by activation, unlike the children with LAD 1. Their neutrophils performed poorly in assays for chemotaxis.<sup>114</sup> These children had less frequent and less severe infections as they grew older and were managed conservatively.

Table 2 Conditions associated with subnormal neutrophil chemotaxis

<i>Defects in the neutrophil</i>
Neonatal neutrophils
Neutrophil actin dysfunction
Syndromes: Schwachman, glycogenosis type 1b, Chediak Higashi, and hyper-Ig E
Acrodermatitis enteropathica (zinc deficiency)
Specific granule deficiency
Chromosome 7 abnormalities
<i>Direct inhibition of neutrophil chemotaxis</i>
Juvenile periodontitis
Immune complex diseases
Wiskott Aldrich syndrome
Bone marrow transplant
Drugs such as corticosteroids, amphotericin B, antithymocyte globulin
Granulomatous diseases such as sarcoidosis
Malignancies
<i>Defective generation of chemotactic factors</i>
Familial complement deficiencies of alternative or classical pathway

### Other disorders of chemotaxis

Normal chemotaxis is essential for the migration of neutrophils from the circulation to the site of infection. It requires the generation of chemotactic substances and normal neutrophilic responsiveness to them. Disordered neutrophil chemotaxis is seen in a wide variety of conditions (table 2); however, in many of these conditions this does not greatly contribute to a decrease in resistance to bacterial infections. When considering disorders of chemotaxis, it must be remembered that laboratory investigations for these are difficult to standardise and subject to artifacts.

*Hyper-IgE syndrome*<sup>115-116</sup>—This condition usually presents in infancy with serious recurrent staphylococcal infections of the skin and the lungs, which sometimes develop into cystic pneumonias. The children may have coarse facies and chronic eczematoid dermatitis. The condition can occur sporadically or as an autosomal dominant condition with variable penetrance.<sup>115</sup> The cause of infections is poorly understood but might be related to the presence of high concentrations of antistaphylococcal IgE, low concentrations of antistaphylococcal IgG, a neutrophil chemotactic defect, or some combination thereof. Recently, Borges *et al* have found that lymphocytes of patients with hyper-IgE syndrome have an impaired response to IL-12, resulting in decreased IFN- $\gamma$  production, and have suggested that a defective IL-12-IFN- $\gamma$  pathway might be of key importance in the pathogenesis of immune abnormalities of the hyper-IgE syndrome.<sup>116</sup> In the absence of a clear understanding of the underlying pathophysiology or of a diagnostic test it is very difficult to know how to draw a diagnostic line between individuals with this syndrome and those with severe atopic eczema and associated staphylococcal superinfection. Blood and sputum eosinophilia may be demonstrated and raised serum IgE, often more than 5000 IU/litre, is found. Concentrations of IgD are also high, whereas those of IgG, IgA, and IgM are typically normal. Lymphocyte counts are normal, but poor antibody and cell mediated responses to antigens are sometimes seen. Variable defects in neutrophil chemotaxis are present in some patients. Prophylactic antibiotics are the mainstay of the management of this disorder. Although many authorities recommend long term penicillinase resistant penicillins, we and others prefer to use cotrimoxazole for its better tissue penetration, reserving antistaphylococcal agents for use against symptomatic infections as indicated. Surgical drainage may also be indicated on occasion. Intravenous immunoglobulin is used in patients with hyper-IgE who are deficient in immunoglobulins or IgG subclasses, or have a known defect in antibody responses to polysaccharide. The administration of IFN- $\gamma$  results in pronounced improvement in neutrophil chemotaxis<sup>117</sup>; however, it is as yet unclear whether such treatment is of any clinical benefit.

## DISORDERS OF OPSONISATION AND INGESTION

Neutrophil phagocytosis is enhanced by opsonisation of the organism by antibody, complement, and mannan binding lectin (MBL). Defects in any of these will impair phagocytosis.<sup>118-119</sup> Measurement of mannan binding lectin, in addition to complement and immunoglobulins, is now undertaken when screening for immunodeficiency. Similarly defects in the neutrophil binding sites for immunoglobulin might predispose to a higher risk of infections by encapsulated organisms. Individuals who are homozygous for both the FcγRIIA allele, with arginine in position 131, and the FcγRIIB\*NA2 allele appear to be at higher risk for meningococcal septicaemic shock<sup>120</sup> and recurrent upper respiratory infections by encapsulated bacteria,<sup>121</sup> owing to defects in IgG2 mediated neutrophil phagocytosis.<sup>122</sup> DNA analysis on blood or saliva can be performed to look for these mutations.<sup>123-124</sup>

## DISORDERS OF DEGRANULATION

The neutrophil granules contain toxic proteins that are released into the phagocytic vacuole to aid microbial killing. The two main conditions associated with defects in degranulation are Chediak Higashi syndrome (CHS) and specific granule deficiency.

*Chediak Higashi syndrome*

CHS is a rare autosomal recessive disorder of granule bearing cells. It is caused by mutations in the lysosomal trafficking regulator gene, *LYST*, present on the long arm of chromosome 1 (1q42.1-q42.2).<sup>125</sup> This leads to defective membrane targeting of the proteins present in secretory lysosomes. There is uncontrolled granule fusion leading to large but defective granules in all granule bearing cells including Schwann cells, melanosomes, and neutrophils. These large granules are seen in both peripheral blood granulocytes and in their progenitor cells in the bone marrow, and are associated with delayed and incomplete degranulation. In addition, these children also have neutropenia (because of the death of many myeloid precursors in the bone marrow) and defective chemotaxis (because intrinsic defects in the neutrophil impair their mobility). Lymphocytes contain large giant cytoplasmic granules and function poorly in antibody dependent cell mediated cytotoxicity of tumour cells. Natural killer cell function is compromised and this may be responsible for the later development of malignancies. A deficiency of granules containing serotonin and adenosine phosphate in platelets leads to defective platelet aggregation and prolonged bleeding time.

Children with CHS present in childhood with partial albinism (hypopigmentation of hair and eyes compared with other family members) and recurrent infections of skin, mouth, and respiratory tract. Death often occurs before 7 years of age because of serious infection or an accelerated phase of the condition, which may be related to infection with Epstein-Barr virus or other lymphotropic viruses, and presents with a lymphoma-like

picture.<sup>126</sup> Individuals who survive to become adults may suffer from neurological disabilities.

The peripheral smear shows large cytoplasmic granules in neutrophils and lymphocytes. Tests for neutrophil function show defective chemotaxis and intracellular killing. Lymphocyte function and platelet aggregation are also abnormal. In the accelerated phase of the illness, there are high and persistent concentrations of antibodies to Epstein-Barr virus antigens. In the stable phase, management is centred around the prompt recognition and treatment of infections. Ascorbic acid appears to correct some of the microbicidal defects *in vitro*, but has not been effective in reducing infections in patients.<sup>127</sup> The management of the accelerated phase is difficult and has included the use of corticosteroids and chemotherapeutic agents. Bone marrow transplantation with HLA matched marrow before the full blown accelerated phase has been successful.<sup>128</sup>

*Specific granule deficiency*

This is an extremely rare, autosomal recessively inherited condition characterised by recurrent bacterial infections starting in infancy associated with absent granules in peripheral blood neutrophils. Management consists of prophylactic antibiotics and aggressive management of infections; individuals have survived into adulthood.

## APPROACH TO CHILDREN SUSPECTED TO HAVE DISORDERS OF PHAGOCYTE FUNCTION

Patients with chronic or recurrent pyogenic or fungal infections and a slow response to antibiotics must be evaluated systematically for phagocyte function using some or all of the following tests.

- (1) A full blood count and peripheral smear examination can provide useful clues to the diagnosis of phagocyte dysfunction. The presence of pronounced polymorphonuclear leucocytosis might suggest the diagnosis of LAD. Neutropenia can be seen in conditions also associated with phagocyte dysfunction such as CHS. The peripheral smear must be examined carefully for features of disorders of degranulation. Specific granule deficiency is characterised by bilobed nuclei in more than 80% of the neutrophils and there is a striking decrease in cytoplasmic granularity. Giant granules are seen in individuals with CHS. MPO deficiency can be diagnosed by the use of appropriate histochemical stains or certain automated cell counters.
- (2) The NBT test and/or quantitative tests to measure respiratory burst activity in stimulated neutrophils using flow cytometric analysis should be performed in children in whom CGD is suspected.
- (3) The measurement of G6PD and glutathione synthetase. Severe deficiency of G6PD or glutathione synthetase can lead to intracellular killing defects similar to CGD and subnormal NBT. In patients presenting with features similar to CGD, these enzymes should be measured if CGD has been ruled out.
- (4) The estimation of CD11/CD18 expression in peripheral blood neutrophils is performed in

infants suspected of having LAD 1. Leucocyte activation before flow cytometry can greatly enhance the expression of these glycoproteins and magnify the differences between healthy subjects and patients.<sup>129</sup> Flow cytometric analysis for SLeX (CD15) expression on leucocytes using an anti-SLeX monoclonal antibody should be performed to rule out LAD 2.

(5) The estimation of serum immunoglobulin isotypes, mannan binding lectin, and total complement haemolytic activity, and DNA studies for Fcγ receptor polymorphisms. Defects in opsonisation caused by deficiencies in these factors are more common causes of disorders of phagocytic function than intrinsic defects in the neutrophil.

(6) Neutrophil chemotaxis can be determined by the use of a modified Boyden chamber.<sup>130</sup> A siliconised or non-siliconised glass tube is used as the upper compartment of the chamber in which neutrophils are placed and fMLP, a potent synthetic chemoattractive peptide, is placed into the lower compartment. The chamber is incubated and the number of cells reaching the bottom surface of the filter is expressed as the average number of cells/field after counting five fields for each filter. Another classic test for the *in vivo* study of adhesion and chemotaxis is the Rebeck skin window.<sup>130</sup> However, these tests are difficult and not often performed outside a research setting. In this regard, it is useful to remember that although subnormal chemotaxis has been described in a variety of conditions, there are no known disorders that can be diagnosed specifically using only functional chemotaxis assays.

(7) Flow cytometric assay of phagocytosis: primary disorders of phagocytosis are rare. As already mentioned, most conditions with subnormal phagocytosis are caused by deficiencies in humoral factors. To look for both intrinsic and extrinsic phagocytic defects, both patient and control neutrophils can be incubated with fluorescein labelled bacteria or particles after opsonisation with patient or normal serum and phagocytosis assessed using flow cytometric techniques.<sup>130</sup>

(8) Bacteriocidal assays using patient cells with either patient or control serum and bacteria such as *S aureus*. Leucocytes from a normal subject phagocytose and kill approximately 95% of the bacteria within 120 minutes. In conditions with intracellular killing defects, such as CGD, the leucocytes kill < 10% of bacteria over this period. Disorders of phagocytosis show viable bacteria outside the cells, whereas in disorders of killing, the viable bacteria are found within the cells. This is a technically difficult assay and, in many centres, other specific assays for the production of reactive oxygen radicals are done directly in children suspected of having disorders of phagocytosis and bacterial killing.

(9) The assessment of adherence of neutrophils to either artificial surfaces or cultured cells is usually undertaken as research rather than a clinical diagnostic procedure.

## Conclusions

Neutrophil disorders are an uncommon, yet important, cause of morbidity and mortality in infants and children and should be considered when investigating children for immunodeficiency. They are especially likely when the clinical presentation includes features such as oral ulcers and gingivitis, delayed separation of the umbilical cord, uncommon infections such as hepatic or brain abscesses, uncommon organisms such as *S marcescens* or *Pseudomonas* spp, or when the individual has features of syndromic conditions associated with neutropenia or neutrophil dysfunction. All patients with recurrent oral infections, skin abscesses, perianal and perirectal abscesses, poor wound healing, sinopulmonary infections, or deep visceral abscesses should be evaluated for defects in phagocyte function. Appropriate investigations can lead to specific diagnoses, and general and specific management measures can reduce both mortality and morbidity and permit genetic counselling and antenatal diagnosis in some cases.

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